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(54) MULTILAYER ANALYTICAL ELEMENTS

- (71) We, EASTMAN KODAK COMPANY, a Company organized under the Laws of the State of New Jersey, United States of America of 343 State Street, Rochester, New York 14650, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—
- This invention relates to a multilayer analytical element, such as an elongated analytical tape, for use in automated spectrophotometric analysis of fluids, and especially of body fluids such as blood.
- In recent years, a number of automated systems for carrying out quantitative chemical analyses of fluid samples have been developed and these have proven particularly advantageous for use in clinical laboratories; especially in the analysis of blood. Systems based on continuous flow analysis in which sample, diluents and test reagents are mixed together and transported through the analyzer are very widely utilized. However, these continuous analyzers, such as, for example, the analyzer illustrated in United States patent 2,797,149, are complex and expensive, require skilled operators, necessitate considerable expenditure of time and effort in repetitive cleaning operations, and do not permit the use of very small quantities of sample, such as are used in microanalytical techniques. In an effort to overcome these disadvantages, automated chemical analyzers have been proposed which utilize a continuous analytical tape on which the sample to be analyzed is deposited and which effect quantitative analysis by means of spectrophotometric measurement of colour-forming reactions between constituents of the sample to be analyzed and test reagents applied to or carried by the tape. Analyzers of this type are described in detail in many patents, for example, in United Kingdom Patent 1,049,364 and in United States Patents 3,036,893 and 3,526,480.
- The analyzers utilizing continuous analytical tapes are inherently much simpler than continuous flow analyzers. However, analytical tape analyzers such as are described in the aforesaid patents suffer from many significant disadvantages which have hindered their commercial development. Thus, where the tape does not incorporate the test reagents within itself and is merely utilized as a means of transporting the sample to be analyzed through the system, as in certain embodiments of United Kingdom patent 1,049,364, provision must be made for separate application of sample and test reagents to the tape at the right time and in the right amounts. The use of separate tapes to accomplish various functions, such as sample spreading, filtering, and reaction of the sample with the test reagents, as in United States patent 3,036,893 and in certain embodiments of United Kingdom patent 1,049,364, adds greatly to the complexity of the system so that the inherent simplicity of the continuous tape method of analysis is not fully realized. Use of analytical tapes of a complex nature which are difficult and expensive to manufacture, such as are described in United States patent 3,526,480 is, of course, also a serious hindrance to achieving a commercially practical system.
- In accordance with the invention, there is provided an integral multilayer analytical element for use in automated spectrophotometric analysis which is simple in structure, easily manufactured at reasonable cost, and adapted to carrying out a variety of different analyses in continuous analyzers in a simple and effective manner. This analytical element is usually used in the form of a long continuous strip or tape and, for convenience in describing the present invention, is generally referred to herein as an analytical tape. However, it will be

apparent that the element can be used in other forms, such as sheets or short strips or in the form of small sections mounted in aperture cards, and all such forms of the element are intended to be within the scope of the present invention. As hereinafter described, the multilayer element incorporates within a reagent layer the test reagents needed for carrying out the analysis so that in use the operator merely needs to provide for proper application of the sample which is to be analyzed. Automated dispensers for applying a controlled amount of sample to the element at the appropriate location are known and any such dispenser may be used with the element of this invention. Quantitative analysis for particular constituents of the sample, for example, analysis for glucose in blood, is readily accomplished by use of conventional spectrophotometers.

The analytical tape of this invention is an integral multilayer tape which provides all the layers needed for carrying out the various functions involved in the analytical process, as contrasted with automated systems of the prior art in which two or more tapes are brought into temporary contact with one another to form a composite structure and then separated. It is comprised of a support, a reagent layer which contains one or more test reagents for reaction with constituents of the sample to be analyzed, and a porous medium, comprised of one or more layers, for performing the functions of spreading and optionally filtering the sample and in some embodiments for facilitating spectrophotometric analysis, as will be hereinafter described in detail. Since the tape is an integral multilayer structure in which the support and the various layers are bonded together and since in use it is only necessary to apply the sample to be analyzed to the top layer of the tape and direct the tape through an appropriate transmission or reflection spectrophotometric analyzer, the equipment used in the automated analysis procedure need not be complex and the inherent simplicity of the continuous analytical tape system of analysis is fully realized.

The support used in the multilayer analytical tape of the present invention is comprised of a light-transmitting, liquid impermeable material. As long as it meets these criteria, the particular material used as the support is not important. A variety of polymeric materials are well suited for this purpose, such as, for example, cellulose acetate, poly(ethylene terephthalate), polycarbonates, or polystyrene. The support may be of any suitable thickness, typically from 0.002 to 0.010 inches thickness.

The reagent layer may be coated directly on the support or a light-transmitting subbing layer may be used to aid in bonding

the reagent layer to the support. The purpose of the reagent layer is to contain the test reagents intended to undergo colour forming reactions with constituents of the sample to be analyzed. A coating of a dispersion of one or more of such test reagents in a hydrophilic colloid which serves as a binder, such as gelatin or polyvinyl alcohol, is suitable as the reagent layer. The particular reagents incorporated in the reagent layer will, of course, be dependent upon the particular material which is to be analyzed and the particular colour-forming reaction that is chosen for identifying this material.

On the side of the reagent layer opposite from the support are located one or more layers which perform the function of spreading the sample to distribute it uniformly in the lateral direction. The same or additional layers may perform the function of filtering the sample to remove components that would interfere with the occurrence or measurement of the colour-forming reaction, and reflecting the light transmitted through the support when carrying out reflection spectrophotometric analysis. Thus, the multilayer analytical tape of the present invention will comprise at least two layers in addition to the support, one of these being the reagent layer and the other being a layer which is capable of performing one or more of the aforesaid functions. However, a separate layer can be used for each of these three functions, if desired, and in this instance the tape would comprise four layers in addition to the support. Alternatively, a single layer can be used to perform two of the three functions and a different layer to perform the third. Also, since more than one layer can be used for a given purpose, for example two or more contiguous layers may be utilized as reagent layers, the multilayer tape can also be of an even more complex construction than the aforesaid four layer embodiment.

The sample spreading layer in the multilayer analytical tape is in the position outermost from the support and is the layer upon which the liquid sample to be analyzed, such as a sample of whole blood, is deposited. Typically, the sample is applied to the sample spreading layer by an automated dispensing apparatus which is capable of dropping a small drop of liquid sample at a desired location. The sample spreading layer functions to distribute the drop uniformly in a lateral direction. Its importance in minimizing the tendency for "ring" formation to occur within the reagent layer of the analytical tape will be apparent from the discussion of this phenomenon in prior patents, such as United States patents 3,036,893 and 3,526,480 which

are referred to hereinabove. Thus, if drops of the sample to be analyzed were applied directly to the reagent layer, in spreading laterally they would form a "ring" in which there is a reduced concentration of test reagent in the central area and an increased concentration about the periphery. This would result in non-uniform colour development which would seriously hinder quantitative measurement by spectrophotometry. However, in the multilayer analytical tape of the present invention, a sample spreading layer is provided in which essentially all lateral movement of the sample takes place so that as sample reaches the reagent layer it does not spread laterally and consequently does not cause uneven distribution of test reagent.

With the multilayer analytical tape described herein, variations in the volume of sample applied to the tape affects the diameter of the coloured spot formed in the reagent layer but the volume of liquid per unit area which reaches the reagent layer is substantially constant regardless of variations in the volume of sample applied. Accordingly, the density of the colour formed in the reagent layer by the colour-forming reaction is not significantly affected by variations in the size of the drop applied to the tape and is dependent only on the concentration of the component undergoing the colour-forming reaction. This makes it unnecessary to apply drops of uniform size to the tape or to know the size of the drop applied in order to obtain the desired quantitative analysis.

The function of the optional filtering layer is to remove from the sample components that are present which would interfere with the colour-forming reaction in the reagent layer or would hinder the spectrophotometric measurements. Thus, in the use of the multilayer analytical tape for analysis of constituents of whole blood, the filtering layer would serve to remove red blood cells while transmitting the serum to the layer below. The filtering layer can be comprised of any material that will provide a proper degree of porosity for the sample being analyzed, with the optimum porosity depending upon the particular use for which the multilayer tape is intended. If the tape is to be used for analysis of whole blood, it is desirable that the filtering layer have a pore size of 1 to 5 microns. This pore size is effective in screening out blood cells, which typically have a size of from 7 to 30 microns, while still providing an adequate rate of passage for the serum. A pore size in the filtering layer which is too small can restrict the utility of the tape, for example, it could unduly inhibit the passage of blood proteins for those tests where it

is important that these be transmitted to the reagent layer.

The multilayer analytical tape of the present invention may be adapted for use in an analytical system employing reflection techniques of spectrophotometric analysis and preferably includes a layer which functions as a reflecting layer and thereby provides a suitable background for spectrophotometric measurement through the support side of the tape. The reflecting layer must be a porous layer to permit the passage of constituents of the sample which are to be analyzed into the reagent layer and should be white in order to provide an effective background for reflection spectrophotometry. In a multilayer tape intended for analysis of whole blood the blood cells are blocked by the filtering layer but they do not interfere with reflection spectrophotometric measurements since these are made through the support with the reflecting layer serving as a suitable white background.

In reading an unused analytical element of the type described herein in the reflection spectrophotometric mode, the photometer-detector sees only about 1% of the energy incident on the element. This 1% of incident radiation then becomes the 100% reference level for subsequent reflection measurements of elements to which a sample has been applied. This low collection efficiency is the result of the diffuse reflectance of the spreading layers of the type described hereinabove, and the small collection angle needed to discriminate against scattered light.

In reading the same element in the transmission mode, the detector sees about 1.5 to 2% of the energy incident on the multilayer element.

Other forms of spectrophotometry can also be used to advantage. These include fluorescence spectrophotometry, wherein the reaction product of the sample and the test reagent is a fluorescent material and reading is with radiation which causes this reaction product to fluoresce.

As hereinbefore described, a single layer can be provided which will serve the functions of sample spreading and filtering and will also serve as a reflecting layer. An example of a suitable layer which will perform all of these functions is a "blush polymer" layer. As is well known, a "blush polymer" layer is a polymer layer formed on a substrate by dissolving a polymer in a mixture of two liquids, one of which is a good solvent for the polymer and the other of which is of higher boiling point and is a non-solvent or at least a poor solvent for the polymer, coating the polymer solution on the substrate, and drying the coating. Since the good solvent will evaporate more readily because of its lower boiling point,

the coating becomes enriched in the liquid which is a poor solvent or non-solvent as evaporation proceeds and, in consequence, the polymer precipitates out in the form of fine particles and forms on the substrate an adherent porous layer. Many different polymers can be used for preparing "blush polymer" layers for use in the invention, typical examples being polycarbonates, polyamides, and cellulose esters.

As an alternative to coating a "blush polymer" layer as described above, a useful layer adapted to perform the functions of sample spreading and filtering, and to provide the necessary reflective background to allow use of the analytical tape in reflection spectrophotometry, can be provided by laminating to the reagent layer a thin layer of a microporous filter membrane. These filter membranes are "blushed polymer" materials, made, for example, from cellulose esters, and contain pores of microscopic size with a variety of materials of differing pore size being available commercially. Examples of materials of this type which are suitable for use in the present invention and which are commercially available are the filters sold under the trademark 'Millipore' by the Millipore Corporation and those sold under the trademark 'Metricel' by the Gelman Instrument Company.

When a single layer is used to serve as a sample spreading layer, the analytical tape can be comprised of only two layers and a support since the only additional essential layer is the reagent layer. However, other layers could also be included, if desired. For example, two or more contiguous reagent layers can be provided to permit successive reactions in each of the layers to occur. Moreover, as hereinbefore described, it is within the scope of the present invention to use one layer which serves as a filtering and reflecting layer and a separate layer to serve as a sample spreading layer or to use three separate layers to carry out the functions of sample spreading, sample filtering and light reflection, respectively. The layers would, of course, be arranged so that the sample spreading layer would be outermost from the support, then would come the filtering layer, the reflecting layer, and finally the reagent layer.

An example of a layer which is useful as both a filtering layer and a reflecting layer is a layer comprised of titanium dioxide or barium sulphate dispersed in a binder such as cellulose acetate, polyvinyl alcohol, or gelatin. This layer is particularly useful in a multilayer tape intended for use in analysis of whole blood since it effectively screens out the blood cells while transmitting the serum and provides an effective white background for spectrophotometric measurement made through the support.

metric measurement made through the support.

A particularly useful layer for use as a sample spreading layer in a multilayer tape intended for use in analysis of whole blood is a layer comprised of a dispersion of diatomaceous earth in a binder such as cellulose acetate. The diatomaceous earth is very effective in distributing the blood uniformly in a lateral direction. Sample spreading layers can also be prepared from microcrystalline colloidal products derived from either natural or synthetic polymeric materials. These microcrystalline materials are described in an article entitled "Colloidal Macromolecular Phenomena, Part II, Novel Microcrystals of Polymers" by O. A. Battista et al published in the Journal of Applied Polymer Science, Vol. II, pages 481-498 (1967). Microcrystalline cellulose, which is commercially available from FMC Corporation under the trademark 'Avicel', is an example of a material of this type which is satisfactory for use in the present invention.

Good results are also obtained with a sample spreading layer comprised of inert spherical particles of uniform size held in a matrix of a binder material which bonds the particles to the underlying layer. Examples of such spherical particles are glass beads and polymeric resin beads. Gelatin and polyvinyl alcohol are particularly good binders for use with the glass beads or resin beads. The binder should be used in a small amount so as to avoid filling any substantial portion of the void volume provided by the spheres. A sample spreading layer comprised of spherical particles of uniform size provides advantages as compared to a porous polymer layer such as a "blush polymer" layer. Thus, it is effective in spreading a drop of blood to a uniform and reproducible area and it does this so rapidly that the spreading is completed before any significant degree of diffusion of blood components into adjacent layers of the multilayer tape can occur. This results in a very uniform concentration of sample constituents in the reagent layer and a uniform color which is measured as a basis for the analysis. While blood cells can cause clogging of porous polymer layers, this does not occur with a sample spreading layer comprised of spherical particles in a matrix of binder. The use of spheres of uniform size provides especially desirable results as it provides an adequate volume of void space while limiting the average dimension of the interstitial spaces to a relatively narrow range. This results in a cessation of spreading of the blood once the interstitial volume in the sample area has been completely filled and also permits rapid drainage of the plasma into the underlying

layer once the spreading process has been completed. Spheres of a size in the range of about 80 to 120 microns are particularly desirable for a sample spreading layer to be used with whole blood. A drop of blood placed on such a layer spreads in only a few seconds to a circle of uniform area and composition and the area covered after spreading is directly proportional to the sample volume. The incorporation of a very small amount of a surfactant in the spreading layer, in addition to the spherical particles, is advantageous as it accelerates the spreading process.

Other layers in addition to those which have been discussed above can also be included in the multilayer analytical tape of this invention. For example, a dialysis layer which is positioned directly over the reagent layer can be provided. This layer would be advantageous in analyzing for glucose in blood as it would permit the passage of glucose into the reagent layer but inhibit the passage of high-molecular-weight proteins which could interfere with the glucose analysis. A semipermeable cellulose acetate membrane which serves to separate high molecular weight materials from low molecular weight materials would be suitable for a dialysis layer. Such a layer functions, of course, by a different mechanism than a porous polymer layer, such as the "blush polymer" layer described hereinabove, which merely acts as a screen in which the pores permit the passage of solutions and small particulate matter but block the passage of particles which are too large to pass through them.

The multilayer analytical tape of the present invention can be adapted for use in carrying out a wide variety of chemical analyses, not only in the field of clinical chemistry but in chemical research and in chemical process control laboratories. It is especially well suited for use in clinical testing of body fluids, such as blood and urine, since in this work a large number of repetitive tests are frequently conducted and test results are often needed a very short time after the sample is taken so that automated testing is highly desirable. In the field of blood analysis, for example, the multilayer tape can be adapted for use in carrying out quantitative analyses for many of the blood components which are routinely measured. Thus, for example, the tape may be readily adapted for use in the analysis of such blood components as albumin, bilirubin, urea nitrogen, serum glutamic-oxalacetic transaminase, chloride, glucose, uric acid, and alkaline phosphatase, as well as many other components, by appropriate choice of test reagents. In analyzing blood with the analytical tape of this invention, the blood cells may first be separated from

the serum, by such means as centrifuging, and the serum applied to the tape. However, it is not necessary to make such separation, as whole blood can be applied directly to the tape and the blood cells filtered out through the action of the filtering layer. The presence of these cells on the tape will not interfere with the spectrophotometric analysis since it is carried out by reflection techniques, with light being transmitted through the support and reagent layer and reflected from the porous reflecting layer. A particularly significant advantage of the analytical tape described herein is its ability to be used to analyze either serum or whole blood.

A wide variety of test reagents can be used in the analytical tapes described herein depending on the component or components of the sample which it is desired to measure and the particular colour-forming reactions utilized. In blood testing, the measurement of glucose can be made by incorporating a ferricyanide compound in the reagent layer and measuring the decrease in the yellow colour of ferricyanide caused by reaction of the ferricyanide with glucose. High molecular weight proteins that are present in blood cause some reduction in ferricyanide and thus will interfere with this test for glucose unless a dialysis layer as hereinbefore described is included in the tape. In testing for uric acid in blood, the reagent layer can consist of a mixture of copper sulphate and neocuproine dispersed in the binder. The uric acid causes the reduction of copper (II) to copper (I) which then complexes with the neocuproine to form a coloured product, with the density of the colour being directly related to the concentration of uric acid. In the determination of the enzyme known as serum glutamic-oxalacetic transaminase, sequential reactions can be used. This enzyme catalyzes the conversion of glutamic acid to oxalacetic acid at a pH of 7.4 and the oxalacetic acid can be measured via coupling with the diazonium salt of a dye known as "Fast Ponceau L". To facilitate the first equilibrium being established before colour coupling, it is desirable to separate the reagents into two distinct layers to provide a suitable time interval for the first equilibrium to be established before being affected by the second reaction. Thus, the glutamic acid should be incorporated in a first reagent layer which is coated over a second reagent layer that contains the salt of Fast Ponceau L.

A typical automated analysis system using the multilayer analytical tape of this invention would provide means for unwinding the tape from a supply roll and for guiding it beneath a sample dispenser where a drop of the sample to be analyzed, such as whole

blood or serum, would be applied to the surface of the tape, then directing the tape through one or more processing zones, and finally winding it on a take-up roll. In the aforesaid processing zones, the tape would be subjected to appropriate conditioning to facilitate the colour-forming reaction between a component of the sample and a component of the reagent layer. Such conditioning could include heating or the application of water or other solvent to promote the reaction. The analytical measurement would typically be made by passing the tape through a zone in which suitable apparatus for transmission or reflection spectrophotometry is provided, such apparatus serving to direct a beam of light through the support which is then transmitted or reflected back respectively to the detecting means. Use of reflection spectrophotometry effectively avoids optical interference from residues, such as blood cells, which have been left on or in the layers of the tape. Conventional techniques of fluorescence spectrophotometry can also be employed if desired. To provide a control for the analysis, a drop of a standard solution of the component which is to be analyzed can be applied adjacent to the area where the drop of sample is placed in order to permit the use of differential measurements in the spectrophotometric analysis.

The multilayer analytical tapes of this invention can be manufactured in any appropriate width, a typical size being a tape with a width of sixteen millimetres, and would typically be produced in the form of a long length of tape wound on a spool or enclosed in a cassette. Automated analytical systems using these tapes can be designed to use only a single type of tape, where the system is intended to carry out a single test on a large number of separate samples, or can be designed to use a number of different tapes, either simultaneously or sequentially, each of which incorporates the reagents needed to analyze for a particular component of the sample. A single analytical tape can be used to analyze for two or more components in a sample by incorporating test reagents for each such analysis in admixture in a single reagent layer or in two or more separate reagent layers as long as the reagents chosen function in such a way that none of them interferes with the functioning of any of the others. The reagent layer can also take the form of a plurality of parallel contiguous stripes of different composition each of which contains test reagents adapted to carry out analysis for a particular component of the sample. As previously disclosed herein, the multilayer analytical element of the present invention can be used in forms other than that of a long continuous tape, for example small sections can be mounted in aperture

cards which are conveyed by appropriate apparatus for handling aperture cards through a sample receiving zone, one or more conditioning zones such as zones for application of heat or solvent, and a zone in which the spectrophotometric measurements are made. A plurality of different analytical elements each of which contains the reagents needed to analyze for a particular component of a sample can be mounted in a single aperture card to enable this card to be used to perform a variety of tests.

In the accompanying drawing, Figures 1 to 4 are enlarged sectional views of multilayer analytical elements of the present invention.

As shown in Figure 1, an analytical element is composed of a support 10, on which is coated a reagent layer 12, a reflecting layer 14 which provides a white background for reflection spectrophotometry through support 10, a filtering layer 16, and a sample spreading layer 18. Reagent layer 12 can be composed of a dispersion of one or more test reagents in a binder such as a gelatin while each of layers 14, 16 and 18 can be a "blush polymer" layer having a pore size adapted to the particular function it is intended to perform. In an alternative embodiment of the invention shown in Figure 2, the analytical element is composed of a support 20 bearing a reagent layer 22 and a layer 24 which serves the functions of sample spreading and filtering and which also provides a suitable background for reflection spectrophotometry through support 20. Layer 24 can be, for example, a "blush polymer" layer which has been coated over layer 22 or a layer of a microporous filter membrane which has been laminated to layer 22. Figure 3 illustrates a further embodiment of the invention in which the analytical element is composed of support 30, reagent layer 32, a dialysis layer 34 which is formed from a semi-permeable membrane and a layer 36, such as a "blush polymer" layer, which serves the functions of sample spreading and filtering and which provides a suitable background for reflection spectrophotometry through support 30. A still further embodiment of the invention is shown in Figure 4 in which the analytical element is composed of support 40, a first reagent layer 42, a second reagent layer 44, a layer 46 which serves as a filtering and light reflecting layer, and a sample spreading layer 48. Layer 46 can be composed, for example, of a dispersion of titanium dioxide in cellulose acetate and layer 48 can be composed of a dispersion of diatomaceous earth in cellulose acetate or of glass beads in gelatin.

The following examples of multilayer analytical tapes as hereinbefore described are given to further illustrate the invention.

EXAMPLE 1

A reagent layer consisting of a dispersion of copper sulphate and neocuproine in gelatin was coated on a 0.004 inch thickness poly(ethylene terephthalate) film at a coating weight of 0.84 mg/dm² of copper sulphate, 1.28 mg/dm² of neocuproine and 53.9 mg/dm² of gelatin. To form a multiplayer analytical tape in accordance with the present invention, there was laminated to this reagent layer a layer of microporous filter material having a thickness of 180 microns and an average pore size of 1.2 microns. The filter material employed was a commercially available cellulose acetate nitrate microporous membrane sold by Millipore Corporation under the trademark 'Millipore' MF filter. It was laminated to the reagent layer by first steaming the reagent layer for several seconds to soften it, then pressing the filter layer into contact with the reagent layer, and then rolling under light pressure to effect bonding. This analytical tape is useful for the detection of uric acid in blood, with the presence of uric acid being indicated by the formation of a pink colour in the reagent layer.

EXAMPLE 2

An analytical tape useful for the detection of uric acid in blood was prepared in the same manner and using the same materials as in Example 1 except that polyvinyl alcohol was used as a binder in the reagent layer in place of the gelatin. The polyvinyl alcohol was employed at a coating weight of 43.1 mg/dm².

EXAMPLE 3

An analytical tape useful for the detection of chloride in blood was prepared in the same manner and using the same materials as in Example 1 except that the reagent layer consisted of silver chromate dispersed in gelatin at a coating weight of 53.9 mg/dm² of gelatin and 18.0 mg/dm² of silver. The presence of chloride in a sample is indicated by this tape by a change in the colour of the reagent layer from reddish-brown to yellow.

EXAMPLE 4

An analytical tape useful for the detection of albumin in blood was prepared in the same manner and using the same materials as in Example 1 except that the reagent layer consisted of 4.66 mg/dm² of hydroxy phenylazobenzoic acid dispersed in 21.0 mg/dm² of a copolymer of acrylic acid and 2-acetoacetoxyethyl methacrylate. The presence of albumin is indicated by the formation of a pinkish-orange colour in the reagent layer.

EXAMPLE 5

A reagent layer consisting of glucose oxidase at 3.15 mg/dm², peroxidase at 3.95 mg/dm², o-dianisidine hydrochloride at 4.42 mg/dm² and gelatin at 215 mg/dm² was coated on a 0.004 inch thickness poly(ethylene terephthalate) film. A slurry was then prepared by mixing 12.0 grams of titanium dioxide and 50 millilitres of a 3% by weight solution of cellulose acetate in acetone and diluting to a volume of 80 millilitres with a mixture of equal parts by volume of acetone and xylene. The slurry was coated over the reagent layer at a coverage of 300 mg/dm² of titanium dioxide and 37.2 mg/dm² of cellulose acetate to form a layer adapted to serve as both a filtering and light reflecting layer. A sample spreading layer was then coated over this layer from a slurry of diatomaceous earth, salicylic acid and cellulose acetate dispersed in a mixture of 1.2 parts dichlorethane to one part acetone by volume. Three different analytical tapes were prepared by utilizing different amounts of diatomaceous earth in the sample spreading layer and each tape was tested for its ability to effectively spread blood by depositing a 10 microlitre sample of whole blood on the sample spreading layer. The time required for the blood to diffuse completely into the tape and the diameter of the spot formed on the tape, as measured through the support, were determined for each or the three tapes. Results obtained were as follows:

Test No.	Diatomaceous Earth (mg/dm ²)	Salicylic Acid (mg/dm ²)	Cellulose Acetate (mg/dm ²)	Diffusion Time (seconds)	Spot Diameter (centimetres)
1	300	3.00	37.2	26	1.05
2	375	3.75	37.2	41	1.00
3	450	4.50	37.2	31	1.00

The analytical tapes described in this example are useful for the detection of glucose in blood, with the presence of glucose being indicated by the formation of a medium brown colour in the reagent layer.

EXAMPLE 6

A reagent layer of the same composition

as that described in Example 1 was coated on a 0.004 inch thickness poly(ethylene terephthalate) film and a layer of microporous filter material as described in Example 1 was laminated to the reagent layer. The layer of microporous filter material was utilized to perform the function of filtering

the sample and to provide a suitable background for reflection spectrophotometry. To form a sample spreading layer, glass beads of 80 to 120 mesh in size, were mixed in a proportion of one gram of beads to 0.5 millilitres of a solution containing 2.5% by weight gelatin and 0.01% by weight of a surface active agent sold by Olin Mathieson Company under the trademark 'Surfactant' 10G (a para-isononylphenoxy polyglycidol having ten glycidol units), and the resulting slurry was spread in a thin layer, such that the amount of slurry containing one gram of beads covered an area of 27 cm², over the layer of microporous filter material. A 10 microlitre sample of whole blood was deposited on the sample spreading layer and spread in less than one second to a circular area of approximately one centimetre in diameter.

EXAMPLE 7

A reagent layer containing glucose oxidase at 2.15 mg/dm², peroxidase at 1.07 mg/dm², o-dianisidine hydrochloride at 3.20 mg/dm² and gelatin at 215 mg/dm² was coated on a 0.007 inch thickness poly(ethylene terephthalate) film. To form a multilayer analytical tape, a layer of the microporous filter membrane described in Example 1 was laminated to the reagent layer. The effect of variation in sample size upon the area and reflection density of the coloured spot formed in the reagent layer was determined by applying different size samples of a glucose solution, containing 100 milligrams of glucose per 100 millilitres of distilled water, to the analytical tape and measuring the area of the spot formed and its reflection density. Readings of the reflection density were taken five minutes after application of the sample to the tape. Results obtained were as follows:

Sample Size (microlitres)	Spot Area (mm ²)	Reflection Density
5	30	0.66
8	50	0.71
10	63	0.70
12	86	0.65
15	92	0.70

As indicated by these results, the reflection density values obtained remain substantially the same as the size of the drop applied is varied. Accordingly, the multilayer analytical tape of the present invention permits quantitative analysis for a component of a sample without it being necessary to apply a known volume of sample to the tape.

EXAMPLE 8

The following solutions were prepared:

(a) 3.23 grams of low temperature setting agarose (polysaccharide) in 70 ml of a 0.3 M buffer of 2-amino-2-hydroxymethyl 1,3-propanediol solution;

(b) 108 international units of glycerol dihydrogenase, 108 mg of human serum albumin, 108 mg resazurin, and 54 mg of diaphorase; and

(c) 54 mg of nicotamide adenine dinucleotide in 10 ml of 0.3 M buffer solution of 2-amino-2-hydroxymethyl 1,3-propanediol.

The foregoing solutions were mixed and coated on a 0.010 inch thickness cellulose triacetate film support to a coverage of 1 square metre. The layer was then laminated with a superposed spreading layer of the type described in Example 1.

Spotting of the surface of this element with aqueous glycerol solutions comprising varying concentrations ranging from about 50 to about 500 mg per decilitre of glycerol produced, after drying, areas of fluorescence which, when observed using a fluorescence spectrophotometer, produced fluorescence proportional to the concentration of glycerol present in the spotted sample solution. Excitation was achieved using radiation of 540 nm wavelength and fluorescence occurred at 590 nm.

EXAMPLE 9

The following solutions were prepared:

(a) 107.6 international units of glycerol dehydrogenase and 107.6 mg of human serum albumin dissolved in 20 ml of 0.3 M glycine buffer (pH 9.6).

(b) 2.150 grams of low temperature setting agarose (polysaccharide) in 70 ml of 0.3 M glycine buffer (pH 9.6).

(c) 504 mg of nicotamide adenine dinucleotide in 10.7 ml of glycine buffer (0.3 M, pH 9.6).

Solutions a, b, and c were mixed at 40°C. and coated on a cellulose triacetate support to form a layer 1 square metre in area. This layer was then laminated with a superposed spreading layer as described in Example 1.

The surface of this element was spotted with varying concentrations of from 50 mg/decilitre of glycerol in water. The spots were permitted to dry for a period of 40 minutes at room temperature. Excitation of the reaction product with radiation of 350 nm wavelength produced fluorescence at 445 nm wavelength. The quantity of fluorescence produced at 445 nm (measured using a fluorescence spectrophotometer) was proportional to the concentration of glycerol present in the test sample.

WHAT WE CLAIM IS:—

1. A multilayer analytical element comprising a liquid impermeable, light-transmitting support carrying at least one reagent-

- containing layer on the whole of one of its surfaces and at least one superposed co-extensive porous layer carried by the reagent-containing layer or layers, the outermost superposed layer being adapted to spread liquid applied to it.
- 5 2. A multilayer analytical element comprising a liquid impermeable, light-transmitting support carrying at least one reagent-containing layer on the whole of one of its surfaces and at least one superposed co-extensive porous layer carried by the reagent-containing layer or layers adapted to spread and filter liquid applied to it and to provide
- 10 a reflective background to the reagent-containing layer or layers for light transmitted through the support.
3. A multilayer analytical element as claimed in Claim 1 or 2 in which the element is in the form of an elongated tape.
- 20 4. A multilayer analytical element as claimed in Claim 1 or 2 in which the element is in the form of a sheet.
5. A multilayer analytical element as claimed in any of the preceding claims in which the support is cellulose acetate, or poly(ethylene terephthalate).
- 25 6. A multilayer analytical element as claimed in any of the preceding claims in which the superposed layer comprises a porous blush polymer layer as herein defined.
- 30 7. A multilayer analytical element as claimed in Claim 6 in which at least one superposed porous layer comprises a plurality of contiguous blush polymer layers having different pore sizes.
- 35 8. A multilayer analytical element as claimed in Claim 2 in which one superposed porous layer comprises a reflective pigment.
- 40 9. A multilayer analytical element as claimed in Claim 8 in which the reflective pigment is titanium dioxide or barium sulphate.
10. A multilayer analytical element as claimed in any of the preceding claims in which the outermost layer comprises a diatomaceous earth or uniformly sized inert spherical particles dispersed in a binder.
11. A multilayer analytical element as claimed in any of the Claims 1 to 9 in which the outermost layer comprises uniformly sized spherical glass beads dispersed in a binder.
12. A multilayer analytical element as claimed in any of the preceding claims in which at least one superposed layer comprises a dialysis layer.
13. A multilayer analytical element as claimed in Claim 12 in which the dialysis layer is a cellulose acetate membrane layer.
14. A multilayer analytical element as claimed in Claim 6 in which at least one superposed porous layer comprises a single blush polymer layer.
15. A multilayer analytical element as claimed in any of the preceding claims in which the reagent-containing layer contains glucose oxidase, peroxidase and o-dianisidine hydrochloride.
16. A multilayer analytical element as claimed in any of the Claims 1 to 14 in which the reagent-containing layer contains copper sulphate and neocuproine.
17. Multilayer analytical element as claimed in Claim 1 and as herein described.
18. Multilayer analytical elements as described in Examples 1 to 9.

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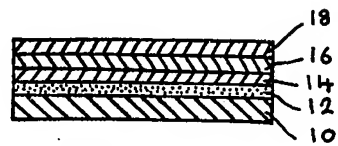


FIG. 1



FIG. 2

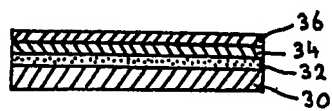


FIG. 3

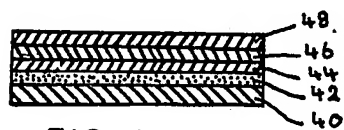


FIG. 4